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The main thrust of this research program was directed towards obtaining a better understanding of the etiology of stress ulceration following thermal injury, ulceration being a frequent and dreaded complication of burns, with a significant mortality.

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STUDY OF MICROCIRCULATORY CHANGES AND CELLULAR DAMAGE ASSOCIATED WITH BURNS

For the Purpose of Improving Therapy for Thermally Injured Army Personnel

(9) FINAL REPORT, 1971-1974

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BY

(10) JOHN C. GAISFORD, M.D.

SUPPORTED BY

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ETIOLOGY OF STRESS ULCER IN BURNED PATIENTS

CONTRACT # DADA17-71-C-1105

DEPARTMENT OF THE ARMY

The main thrust of the research programs supported by the United States Army contracts at the Burn Research Center of The Western Pennsylvania Hospital has been directed toward obtaining a better understanding of the etiology of stress ulceration following thermal injury. Such ulceration, with or without hemorrhage, is a feared complication of burns, with an incidence as high as 12% in one series[11] and a significant mortality.

Since the etiology of stress ulceration was unknown despite intensive research, and since thermal injuries among military personnel are more frequent than among civilians, both at war and in peace time, the Unites States Army has subsidized our research program.

It is our belief that the variable rates of gastric acid secretion,[3] mucous production and steroid elaboration[10,15] following burns, as well as the inverse relationship between the rate of gastric secretion and burn size,[6] were all related to a mucosal injury. The contrast between gastric secretion in the burned patient[6] and our studies of hypersecretion in unburned patients[5] was striking, and reinforced our belief that a mucosal injury occurred in burned patients, most likely related to changes in gastric blood flow.

It was our decision to evaluate altered vascular flow to the stomach at both the microcirculatory level and the macrocirculatory level. As previously reported, the gastric microcirculation was studied by injecting opaque material (Microfil) into the vessels of burned and unburned animals. After clearing of

the specimens and study of the vessels, we found that marked arteriovenous shunting occurred following burns, thus confirming our hypothesis of a vascular etiology of the changes in the physiology of the mucosal cells.

Macrocirculatory studies were then undertaken to determine changes in blood flow to the abdominal viscera. Statham Lab flow probes were used to quantitate celiac and superior mesenteric arterial flow. It had been our intention to chronically implant these probes and then longitudinally study the flow changes in animals following a standard burn. The rabbit turned out to be a poor animal for this type of study because of infection and probe destruction. Slight angulation of these small probes resulted in marked fictical changes in blood flow.

Consequently, flow (cc./min./kg.) was determined in a control series of animals, as well as animals at 24, 48, 72 and 96 hours post standard burn. All animals were anesthetized, probes were inserted , stabilization accomplished, and readings then obtained for two hours.

We found that in control animals there is equal flow in the celiac and superior mesenteric arteries. At 24 hours post burn, there is a 40% reduction of the flow in the superior mesenteric artery and a 60% reduction in the celiac blood flow. At 48 hours the reduction in the superior mesenteric flow is 20% of normal, and at 72 and 96 hours, there is no statistical difference between post burn animals and controls. Celiac flow is reduced to 50% of normal at 48 hours and to 30% of normal at 72 hours and 96 hours.

We thus demonstrated profound macrocirculatory changes to the abdominal viscera, the most profound changes being to the circulation of the stomach and liver. Unfortunately, the small caliber of the gastric and hepatic arteries did not permit differential upper abdominal blood flow studies.

We were struck by the findings of Chiu and his associates[1], that less adenosine triphosphate is present in the mucosa of scalded animals 24 hours following burns. We therefore set out on an ambitious program of quantitative analysis of adenosine triphosphate production, and of enzyme staining of gastric and duodenal mucosa so as to study changes in alkaline phosphatase, acid phosphatase, leucine aminopeptidase, succinic acid dehydrogenase and diphosphopyridine nucleotide diaphorase.

We wished to perform longitudinal studies of the adenosine triphosphate content of the gastric mucosa as Chiu[1] had done at 24 hours post standard burn, so that we could determine the time of maximum "energy reserve" depletion. The method of Forte[2] was used for these determinations. We could not consistently reproduce Chiu's results despite our ability to accurately perform the ATP assay. This surely is a result of the mucosal scrape technique described by Chiu in an attempt to remove the mucous and relate findings to cellular weight only.

Identical histochemical studies of gastric and duodenal mucosa were performed on specimens from control animals and animals at 24, 48, 72 and 96 hours post standard burn. Routine hematoxylin and eosin stains were also performed.

We stained these specimens for intracellular alkaline phosphatase and leucine amino peptidase to determine alterations in cellular brush border function. The method of Pryse-Davies[13] was used for the alkaline phosphatase staining and resulted in good enzyme localization for the specimens in which extensive mucosal slough had not occurred.

Similarly, by using the method of Nachlas[8], leucine aminopeptidase was localized in the brush border. Subjectively, less of both of these enzymes was present at 24 and 48 hours, while regeneration of the enzymes appears to begin at 72 hours and continue in the 96 hour specimens.

Quantitative mitochondrial function was assayed histochemically by staining for succinic acid dehydrogenase and diphosphopyridine nucleotide diaphorase. The methods described by Pearse[12] and Myren[7] were used for succinic dehydrogenase and were most successful. There was a marked decrease in enzyme at 24 and 48 hours, with minimal regeneration at 72 hours and moderate reconstitution of enzyme at 96 hours.

Diphosphopyridine nucleotide diaphorase was localized, using the method of Scarpelli[14], and resulted in findings of mitochondrial function as noted using the succinic dehydrogenase stains.

Lysosomal function was to be studied by determining intracellular acid phosphatase content. Attempts to stain for this enzyme by the methods of Pryse-Davies[13] and Gomori[4] were totally inconsistent due to lack of staining or stain precipitation.

Standard hematoxylin and eosin slides demonstrated marked mucosal changes following our standard burn. The cellular architecture was less

distinct and there was a definite tendency toward mucosal slough in the 24 and 48 hour specimens.

Because of the fragility of the mucosa post burn, consistent results using histochemical techniques cannot lead to quantitative measurements that permit statistical analysis. Perhaps the only present exception to that is measurement of succinic dehydrogenase by the "Swiss Roll" method of Pearse[12] which Myren[7] so elegantly used for correlating acid secretion with succinic dehydrogenase function. Alkaline phosphatase and particularly acid phosphatase stains are so unstable and pH sensitive that quantitative staining is impractical.

Despite the technical difficulties which we encountered, the subjective changes noted in alkaline phosphatase, leucine aminopeptidase, succinic dehydrogenase and diphosphopyridine nucleotidase, are a further indication of the marked intracellular gastric and duodenal mucosal changes that occur following significant thermal injury.

CONCLUSIONS

1. Thermal injury results in microvascular mucosal alterations with venospasm and arteriovenous shunting.
2. Thermal injury results in macrovascular changes with a decrease in abdominal visceral flow, most marked and prolonged in flow to the upper abdominal viscera.
3. The expected and previously reported decrease in gastric and duodenal adenosine triphosphate production could not be confirmed.

4. A subjective decrease occurred in the mucosal intracellular alkaline phosphatase and leucine aminopeptidase content following thermal injury. Enzyme reconstitution appears to begin at 72 hours post burn and reflects cellular brush border function.
5. Succinic dehydrogenase and diphosphopyridine diaphorase content of mucosal cells is semiquantitatively decreased at 24 and 48 hours, with minimal regeneration at 72 hours.
6. Acid phosphatase histochemistry is subject to minute variations in technique and uses unstable buffers and stains that make it unreliable for even semiquantitative use.

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